

10/721,047

~~Divisional of Appl. No. 09/513,907~~
Amdt. dated 11/21/2003
Preliminary Amendmen

PATENT

Amendments to the Specification:

Please replace the paragraph beginning on page 2, line 5 with the following paragraph:

--This application claims priority to copending U.S. Patent Application Serial No. 09/513,907, filed on February 25, 2000, which claims priority to U.S. Provisional Application Serial No. 60/130,238, filed on April 20, 1999. U.S. Patent Application No. 09/513,907 is also related to U.S. Provisional Patent Application 60/075,715, filed on February 24, 1998, U.S. Patent Application Serial No. 09/513,486 (now U.S. Patent No. 6,537,432), filed on February 25, 2000, and U.S. Patent Application Serial No. 09/513,395 (now U.S. Patent No. 6,379,971) filed on February 25, 2000. All of these applications are herein incorporated by reference in their entirety for all purposes.--

LC
10/30/06
Please replace the paragraph beginning on page 60, line ⁵~~7~~ with the following paragraph:

--In some instances, the proteins separated by the methods of the invention are subjected to further analysis by mass spectroscopy. In such instances, particular labels can be utilized to enhance separation of mass fragments into certain parts of the mass spectrum. Suitable labels in such methods are set forth more fully in U.S. Patent Application Serial No. 09/513,395 (now U.S. Patent No. 6,379,971) filed on February 25, 2000. This application is incorporated herein by reference in its entirety.--

Please replace the paragraph beginning on page 63, line ¹⁵~~10~~ with the following paragraph:

--The methods of the invention need not end with the last electrophoretic method of the series. As illustrated in FIG. 1, resolved proteins can be further analyzed by non-electrophoretic methods. Examples of such methods include infra-red spectroscopy, nuclear

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magnetic resonance spectroscopy, UV/VIS spectroscopy and complete or partial sequencing. Coupling the current electrophoretic-based method to various mass spectroscopy (MS) methods is one specific example of further analysis that can be conducted. A variety of mass spectral techniques can be utilized including several MS/MS methods and Electrospray-Time of Flight MS methods (*see, e.g.*, [61], [62], [63], and [64]). Such methods can be used to determine at least a partial sequence for proteins resolved by the electrophoretic methods such as a protein sequence tag (for a discussion of protein sequence tags, *see, e.g.*, [65] and [66]). Further discussion regarding combining the electrophoretic separations described herein with mass spectral analysis is set forth in U.S. provisional application 60/130,238 entitled "Rapid and Quantitative Protein Expression and Sequence Determination," filed April 20, 1999, and to which this application claims benefit and which is incorporated by reference in its entirety. Other mass spectral methods that can be combined with the methods of the present invention are described in U.S. Patent Application Serial No. 09/513,486 (now U.S. Patent No. 6,537,432), filed on February 25, 2000, both being incorporated by reference in their entirety.--

10/30/06
Please replace the paragraph beginning on page 89, line 26 with the following paragraph:

--The methods of the present invention are utilized in order to determine the sequence of a polypeptide. Within preferred embodiments of the invention, the polypeptide is "substantially pure," which means that the polypeptide is about 80% homogeneous, and preferably about 99% or greater homogeneous. Many methods well known to those of ordinary skill in the art may be utilized to purify the polypeptide prior to determining its amino acid sequence. Representative examples include HPLC, Reverse Phase-High Pressure Liquid Chromatography (RP-HPLC), gel electrophoresis, chromatography, or any of a number of peptide purification methods (*see, generally* the series of volumes entitled METHODS IN PROTEIN SEQUENCE ANALYSIS). Even more preferred is the use of capillary electrophoresis and particularly, multi-dimensional capillary electrophoresis, such as that

Amendments to the Specification:

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10/30/06
Please replace ~~paragraph bridging pages 10 and 11~~ ^{paragraph beginning at page 11, line 3} with the following amended paragraph:

For example, in the mass spectrum of a 1425.7 Da peptide (HSDAVFTDNYTR (SEQ ID NO:1)) isolated in an MS/MS experiment acquired in positive ion mode, the difference between the full peptide 1425.7 Da and the next largest mass fragment (y_{11} , 1288.7 Da) is 137 Da. This corresponds to the expected mass of an N-terminal histidine residue that is cleaved at the amide bond. For this peptide, complete sequencing is possible as a result of the generation of high-abundance fragment ions that correspond to cleavage of the peptide at almost every residue along the peptide backbone. The generation of an essentially complete set of positively-charged fragment ions that include either end of the peptide is a result of the basicity of both the N- and C-terminal residues (H and R, respectively). If a basic residue is located at the N- or C-terminus, especially R, most of the ions produced in the CID spectrum will contain that residue since positive charge is essentially localized at that site. This greatly simplifies the resulting spectrum since these basic sites direct the fragmentation into a limited series of specific daughter ions. Peptides that lack basic residues tend to fragment into a more complex mixture of fragment ions that makes sequence determination more difficult.

Please replace the second paragraph on page 105 with the following amended paragraph:

The deduced N-terminal amino acid sequence of glycogen phosphorylase A from Example 1 (*i.e.*, SRPLSD (SEQ ID NO:2)) was used to search the SWIS-PROT and TrEMBL protein sequence databases using the published Expasy TagIdent tool (*see*, <http://www.expasy.ch/tools/tagident.html>). This tool enables searching known protein sequences contained within the database for any that contain matching sequences to a 1-6 contiguous amino acid PST. The search can be limited by the position of the PST in the protein (*i.e.*, N-terminal or C-terminal) and the use of the electrophoretic coordinates isoelectric point and/or apparent molecular weight.